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Stores to Die For

Genes that regulate apoptosis are well defined. In contrast, it has not been clear what genes are central to necrotic cell loss. In the September 27th issue of *Neuron*, Xu et al. (2001) report a critical role for genes that regulate storage and release of Ca^{2+} from the endoplasmic reticulum as important to necrotic-like cellular degeneration in *Caenorhabditis elegans*.

During development and under pathological conditions, cell loss is classically characterized as proceeding through one of two morphologically distinct types of cell elimination (Kerr et al., 1972). One pathway, programmed cell death or apoptosis, has been well elucidated and is typically characterized by condensation of the doomed cell and its organized disruption into apoptotic bodies. Apoptosis is a common feature of development and normal tissue homeostasis, and requires defined gene activities, many of which were elucidated through the powerful genetic approaches of the nematode *C. elegans* (Metzstein et al., 1998; see Figure). In contrast, it has not been clear whether specific gene activities are involved in the process of necrotic cell death, which typically occurs in pathological situations and is characterized by cellular swelling and rupture of organelles and membranes. Now, through *C. elegans* genetics, genes involved in necrotic-like cell death have been defined. Interestingly, these genes reinforce a central role of calcium—a long recognized player in ischemic and excitotoxic injury (Choi, 1988)—and suggest that simply lowering Ca^{2+} storage levels in cells affords protection.

In *C. elegans*, neuronal loss due to select dominant mutations in the *mec-4* gene occurs by necrotic-like death based on morphological and genetic criteria; the neurons swell in the process of degeneration, and death is independent of initiation and execution genes that define programmed cell death, such as *ced-3* caspase (Driscoll and Chalfie, 1991; Chung et al., 2000). The *mec-4* gene encodes a degenerin Na^{+} channel; select gain-of-function alleles that induce necrotic loss of neurons are thought to result in excessive or toxic ion influx. Reported in the latest issue of *Neuron*, K. Xu, N. Tavernarakis, and M. Driscoll have now performed a genetic screen in order to define molecular mechanisms of *mec-4(d)*-induced necrotic-like cell death.

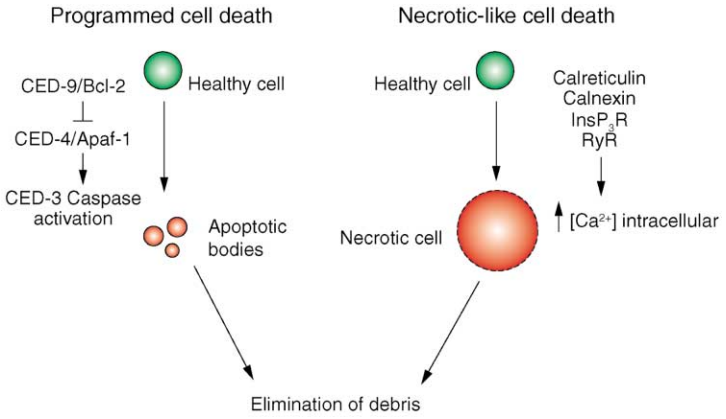
A number of recessive suppressor mutations were identified that restore normal motility to animals paralyzed by a transgene driving ectopic expression of a *med-4(d)* mutant channel in cells of the ventral nerve cord. One complementation group, comprised of four distinct mutations, revealed the involvement of the *calreticulin* gene.

What is calreticulin and how might loss of its activity suppress necrosis? Calreticulin is an abundant, widely expressed protein found predominantly in the lumen of the ER, with both chaperone and Ca^{2+} binding properties (Corbett and Michalak, 2000). RNAi disruption of calnexin, which shares sequence homology with calreticulin but is located in the ER membrane, also protects against *mec-4(d)* toxicity, emphasizing the shared functions of these proteins. Part of the suppression can be attributed to simply lowered levels of the MEC-4 protein itself. MEC-4 is thought to be glycosylated and presumably processed through the ER, and mutation in *calreticulin* apparently affects biosynthesis of the channel, thereby providing one means to reduce toxicity, namely, to reduce levels of the toxic protein.

But what about the intriguing possibility of a role for calreticulin in ER Ca^{2+} homeostasis? Calreticulin was first identified as a major Ca^{2+} binding protein, and Xu et al. (2001) provide evidence that calreticulin function is required within cells expressing the *mec-4(d)* transgene. One can thus imagine that, in the absence of calreticulin, less Ca^{2+} might end up stored in the ER to play havoc upon toxic insult, thereby resulting long term in neuroprotection. And surprisingly, mutation of *calreticulin* in *C. elegans* has generally modest effects on development and behavior.

To address a role of intracellular Ca^{2+} regulation by the ER in *mec-4(d)*-induced neurodegeneration, Xu et al. (2001) used a variety of techniques, including genetic and pharmacological approaches. Calcium is released from ER stores by the activities of the inositol triphosphate receptor ($InsP_3R$) and ryanodine receptor (RyR) Ca^{2+} channels. Mutations in these genes also reduce *med-4(d)*-induced cell swelling. Moreover, long-term treatment with Ca^{2+} chelators and dantrolene, a drug which blocks release of ER Ca^{2+} stores, partially suppresses *mec-4(d)* toxicity. Thapsigargin, a drug that inhibits Ca^{2+} reuptake by the SERCA ER Ca^{2+} pump, attenuates suppression conferred by mutation of *calreticulin*. Indeed, thapsigargin treatment alone induces cell swelling reminiscent of *mec-4(d)* neurodegeneration. These combined data support the idea that elevated intracellular Ca^{2+} from ER stores contributes to necrotic cell death induced by *mec-4(d)* activity.

Additional evidence supports a broad role for calreticulin in necrotic-like death that occurs in *C. elegans*. Other insults, including hyperactivating mutations in additional degenerin channel family members and ectopic



Genes Involved in Programmed Cell Death and Necrotic-like Cell Death, in *C. elegans*

Programmed cell death occurs normally during development and is part of general tissue homeostasis, whereas necrosis is associated with swollen and ruptured cellular membranes and organelles, and is typical of injury or excitotoxicity. In *C. elegans*, the genes involved in initiation and execution of programmed cell death are *ced-9*, *ced-4*, and *ced-3* (Metzstein et al., 1998). Necrosis is characterized in *C. elegans* by visible cellular swelling and lysis, and, when appropriate, loss of cellular function. Necrotic-like death occurs even when programmed cell death is blocked by mutation. Genes defined by Xu et al. (2001) that are critical for necrotic-like cell death caused by ectopic expression of the toxic *mec-4(d)* transgene influence Ca^{2+} storage in or Ca^{2+} release from the ER, suggesting that elevated intracellular Ca^{2+} from ER stores are part of the necrotic death process. Mutational loss of calreticulin, calnexin, or the ER Ca^{2+} channels $InsP_3R$ and the RyR leads to protection from degeneration induced by *mec-4(d)* expression. Common genes are used to clear the cellular debris from both types of cell loss (Chung et al., 2000).

expression of an activated $G\alpha_s$ protein (Berger et al., 1998), cause necrotic-like death that can also be suppressed by mutation of the *calreticulin* gene. However, consistent with a critical role for the Ca^{2+} binding properties of calreticulin in this necrosis, bypassing the ER as a source of toxic intracellular Ca^{2+} with a mutation that induces necrosis by excessive Ca^{2+} influx from the plasma membrane (Treinin and Chalfie, 1995) fails to be suppressed upon loss of *calreticulin*.

Despite this genetic affirmation of a central role of intracellular Ca^{2+} from the ER as critical to necrosis, many questions remain. The processes involved have short-term kinetics, yet the treatments used to interfere with the necrotic-like death were provided long term, scored in progeny treated from very early on. Will acute treatment have an effect? If not, why is such long-term treatment necessary? No other manipulation that Xu et al. (2001) performed matches the degree of neuroprotection associated with mutational loss of the *calreticulin* gene. This may be due to experimental limitations such as efficacy of drug delivery, but might also reflect additional functions of *calreticulin*. Indeed, two of the suppressor mutations (albeit partial suppressors which are effective against transgenically expressed, but not endogenous, *mec-4(d)* activity) are missense mutations that fall within the N-terminal domain of calreticulin, best known for interactions with other ER chaperone proteins (Corbett and Michalak, 2000), and not within the Ca^{2+} binding domains. And, of course, what are the next steps—what cellular processes and specific genes are the targets of Ca^{2+} in the necrotic process?

These studies open new paths and leave us with the promise of things to come. Knowing the central role of specific genes in necrotic-like cell death in *C. elegans* provides better tools to apply toward the understanding of what type of cell death is occurring under different circumstances. Do the same genes contribute to necrosis in other systems, and what is their involvement in other types of cellular degeneration that cannot be clearly classified as necrotic or apoptotic? As noted, pharmacological agents are available to interfere with ER Ca^{2+} homeostasis, and some evidence suggests that dantrolene treatment affords protection in mammalian models for necrotic cell damage. Still, care must be taken when manipulating intracellular Ca^{2+} levels, as this might have deleterious effects on many pathways. The studies by Xu et al. (2001) provide a starting point to define the pathway of cell loss by necrosis.

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